of recurrent disease). Moreover, molecular imaging can aid in the different steps of the drug development process speeding up drug development and validation.
In disease staging for instance, PET has been proven to have high accuracy in detecting unsuspected but pathological lymph nodes and other metastases, and this has been further improved with the use of integrated PET/CT systems. Precise and accurate target delineation is the first step in delivering curative doses of radiation while sparing surrounding normal tissue. Images from specific tracers can assist normal treatment planning and allow dose painting of radioresistant foci to improve biological dose conformality. In addition to selectively targeting subregions within the tumor with higher doses, tumor specific therapies including molecular targeted therapeutics may be incorporated into treatment. This approach is currently being pioneered using specific tracers to image hypoxia, but has broader implications, such as targeting rapidly proliferating areas within tumors or areas expressing other forms of molecular heterogeneity. As a response indicator, volume measurement is known to lack specificity and significance. PET/CT/MRI of functional parameters can assist in assessing outcome and can also help differentiate viable tumor from treatment-induced effects such as fibrosis, atelectasis, and radiopneumonitis. The best tracers and optimal timing of these exams before, during and after treatment is still under experimental investigation and before PET/CT/MRI imaging enters into the clinical routine of the oncology department, several methodological issues need to be addressed. For example, PET-based target volume definition using different PET tracers needs to be studied. Finally there is an urgent need for controlled studies to establish the impact of PET/CT/MRI on the final outcome of patients treated by molecular imaging guidance.

40 INVITED

Targeting tumour cells

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Radiotherapy is highly effective to inactivate clonogenic tumour cells. While untreated tumours contain a large number of clonogenic tumour cells, recurrences after high dose radiotherapy originate from a few surviving clonogenic tumour cells. Based on radiobiological considerations, additional cell kill among the survivors would result in a substantial increase in local tumour control probability. Additional cell kill can be achieved by different approaches including radiation dose escalation, combination with cytotoxic chemotherapeutics and biological targeting compounds. In the clinical situation, radiation dose escalation and intensification of chemotherapy is often limited because of normal tissue complications. Biological targeting compounds in the context of radiotherapy are specifically designed to modify functions relevant for radiation response in either malignant (direct targeting) or non-malignant (indirect targeting) cells in tumour tissues. As a monotherapy these targeting compounds have only a modest anti-tumour efficacy but in combination with radiotherapy results from preclinical and clinical investigations are very promising. Important examples for direct targeting compounds are EGF receptor inhibitors and for indirect targeting anti-angiogenic agents. In principle, both targeting approaches were shown to be effective in combination with fractionated radiotherapy. However, further investigations into molecular and cellular mechanisms of interaction are necessary to better define and exploit the potential of biological targeting of tumour cells to improve outcome after radiotherapy.

41 INVITED

Imaging of the microenvironment

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New strategies that have improved the outcome of head and neck cancer include altered radiotherapy schedules, combination of radiotherapy with chemotherapy, hypoxic sensitizers and, more recently, with EGF receptor inhibitors. These treatments target one or multiple of the major radiation resistance mechanisms: intrinsic radiosensitivity, tumor cell proliferation and hypoxia. Notwithstanding their succes, only a minority (15% at best) of the head and cancer patients profit from each of these new treatment strategies whereas all of them experience the increased toxicity which often is not insignificant. Furthermore, head and neck cancer is a heterogeneous disease and patient selection based on the traditional clinical and histopathological characteristics is not successful. Methods for qualitative and quantitative assessment of functional microenvironmental parameters such as hypoxia and tumor cell proliferation have identified several candidate markers for future use in predictive assays. Before these molecular markers qualify for application in routine clinical practice, they must be validated against reference tests of proliferation and hypoxia and their potential should be demonstrated in well-designed prospective studies. This overview will address the progress in this field of research and discuss a number of promising markers and marker profiles currently under investigation. In conclusion, there have been important gains in the treatment of head and neck cancer in the last decade but there is a need to apply the new treatments more effectively. Identification of biological tumor characteristics may allow a better selection of patients for intensified treatments. The ultimate aim is to provide the best attainable quality of life for individual patients and the cancer patient population as a whole and to apply new therapies in a cost-effective manner.

42 INVITED

Targeting the microenvironment

M. Brown. *Stanford University Medical Center, Department of Radiation OncologyCCSR South Room 1255, Stanford, USA*

Seminal publications in the early 1950s by Gray, Thomlinson and colleagues alerted the scientific community to the possibility that solid tumors contained cells at low oxygen concentrations and that, because of their resistance to killing by radiation, these hypoxic cells could adversely affect the curability of patients by radiotherapy. These predictions have proven correct: Today it is widely accepted that the majority of human tumors have viable hypoxic cells, and that these affect sensitivity to radiotherapy and to chemotherapy, provide a major angiogenic stimulus and increase the probability of metastasis. However, despite more than 50 years of clinical experimentation, we still do not have a proven, effective solution for overcoming the radiation resistance conferred by tumor hypoxia. This is the problem of tumor hypoxia. But there is also a opportunity: Tumor hypoxia could be an advantage in cancer treatment: It is a unique feature that can be targeted by appropriate hypoxia-activated drugs. Will this become a clinical reality with hypoxia activated cytotoxins such as tirapazamine, and PR-104? Meanwhile we are now much more aware of the fact that tumors comprise large numbers of normal, host-derived cells, and that this so-called tumor stroma, particularly the vasculature, is a crucial requirement for tumor growth and a potential target in cancer treatment. Indeed recent data from several groups have suggested that hypoxia confers tumor resistance to radiation by protection of the vasculature by hypoxia inducible factor (HIF-1) mediated pathways distinct from the classical oxygen effect of radiobiology. How much do these pathways contribute to tumor radiation resistance, and can the HIF-1 pathway be exploited by making the tumor vasculature more vulnerable in radiotherapy? In this lecture I will attempt to shed light on these questions.

Symposium (Mon, 24 Sep, 14:45−16:45)
Invasion and metastases

43 INVITED

Genome and transcriptome analysis of single disseminated cancer cells

C. Klein. *University of Regensburg, Abteilung für Onkogenomik, Regensburg, Germany*

Background: It is obvious that later arising metastases are derived from one or several tumor cells that disseminated prior to surgical resection of the primary tumor. Indeed, single disseminated cancer cells residing in various organs after so-called “curative” surgery can be detected by sensitive molecular and immunocytochemical assays. Clinical follow-up studies have established the prognostic significance of disseminated tumor cells for many types of carcinomas, although they are detected in bone marrow or lymph nodes at a frequency of only 10−5 to 10−6. Materials and Methods: The prognostic impact of disseminated tumor cells (DTC) suggests that they are likely candidates for metastatic progenitor cells and that they are important target cells of adjuvant therapies. Unfortunately, only circumstantial knowledge about these cells is currently available. Therefore, we started to develop techniques for the study of single cells and to investigate the early stages of systemic tumor progression. Thus far, we succeeded in establishing protocols for the isolation of DTCs by micromanipulation as well as single cell genome and transcriptome analysis. Results: Results obtained from the genome analysis of several hundred samples of cancer patients demonstrate that dissemination is an early event in the genomic development of a tumor and suggest a parallel evolution of the primary tumor and its metastases. Phenotypic characterization of single disseminated cancer cells identified several subsets of disseminated cancer cells. A comparative analysis of primary tumors and DTCs revealed that important therapy target genes are not equally expressed and genetically activated in local and systemic disease. Conclusions: Metastatic precursor cells are genetically heterogeneous and

different from primary tumors, but harbor frequently altered chromosomal regions. The data emphasize the need to directly analyze the target cells of adjuvant therapies and indicate that the development of a novel pathology for minimal systemic cancer may overcome the limitations inevitably linked to conventional diagnostic studies of the primary tumor.

44 INVITED

Invasive growth: a MET-driven genetic programme for cancer and stem cells

C. Boccaccio. *University of Turin, Institute for Cancer Research and Treatment, Candiolo, Italy*

Invasive growth is a genetic program in which cell proliferation combines with cell-cell dissociation, migration and protection from apoptosis. It occurs under physiological conditions –during development of epithelial organs, angiogenesis, wound healing- and in cancer progression towards malignancy. Recent evidence suggests that, in post-natal life, invasive growth is a program for stem and progenitor cells. This program is triggered by MET, a proto-oncogene whose expression is regulated by self-renewal signals and by unfavourable microninvironment conditions, such as hypoxia, which typically occurs during tissue regeneration and tumour growth. MET encodes a tyrosine kinase receptor that, upon activation by its ligand HGF (a protein closely related to blood coagulation factors), induces cell motility and displacement toward more favourable tissue environments. Interestingly, MET activation turns on hemostasis genes, promoting a thrombohemorragic phenotype in the mouse, which resembles the Trousseau's syndrome observed in cancer patients. Hemostasis activation ends up in peritumoral fibrin deposition. Fibrin acts as a quick-setting extracellular matrix that promotes angiogenesis and offers anchorage for cancer cell migration and intravasation. The MET oncogene thus provides a functional mechanistic link between tumor hypoxia, hemostasis activation and invasive growth.

45 INVITED

Inhibition of hypoxia-induced lysyl oxidase prevents metastasis

A. Giaccia, J.T. Erler. *Stanford University Medical Center, Center for Clinical Science researchRoom 1250, Stanford, USA*

Cancer progression involves the spread of tumor cells through the body. Understanding this process (metastasis) is invaluable, particularly in metastatic breast cancer which is currently incurable. Tumors contain areas of low oxygen (hypoxia) because cells grow and divide faster than blood vessels can provide oxygen. Cells that exist in these areas are highly aggressive and prone to metastasis, although the reasons for this are unknown. All solid tumors contain areas of low oxygen tension (hypoxia). Hypoxic cells are highly aggressive and metastatic, though the underlying processes remain unclear. We report that lysyl oxidase (LOX), an enzyme essential for the formation of the extracellular matrix, is increased by hypoxia. Inhibition of LOX expression/activity prevents in vitro invasion of human breast and cervical cancer cells and in vivo metastasis. Orthotopically grown breast cancer tumors demonstrates co-localized protein expression of LOX and hypoxia. Estrogen receptor negative breast cancer patients with high LOX expressing tumors, have poor distant recurrence-free metastasis and overall survival. These findings indicate LOX increases the metastatic potential of hypoxic human breast cancer cells, and that inhibition of LOX prevents development of metastases, and thus provides a novel therapeutic target.

46 INVITED

The role of tumour-associated macrophages in tumour angiogenesis and metastasis: regulation by hypoxia and angiopoietin-2

C.E. Lewis. *University of Sheffield Medical School, Academic Unit of Pathology, Sheffield, United Kingdom*

Monocytes are recruited into tumors from the circulation, differentiate into tumor-associated macrophages (TAMs) and promote tumour angiogenesis and metastasis. Our recent data has shown that exposure to two signals in the tumour microenvironment; hypoxia (low oxygen) and the cytokine, angiopoietin-2 (Ang-2), helps to activate this aggressive pro-tumour phenotype in TAMs. These cells accumulate in hypoxic areas in tumours where they are exposed to tumor hypoxia and undergo marked phenotypic changes. These include the upregulation of a number of hypoxia-inducible transcription factors like HIFs 1 and 2, which in turn activate the expression of a wide array of genes encoding mitogenic, pro-angiogenic and pro-metastatic cytokines and enzymes. As hypoxia is a hallmark feature of malignant tumors and hypoxic tumor cells are relatively resistant to radio- and chemotherapy, these areas have become a target for novel forms of anticancer therapy. We therefore developed a form of hypoxia-targeted gene therapy in which macrophages are armed with therapeutic genes or viruses that are activated by hypoxia-responsive promoter elements. This restricts transgene expression/viral replication to hypoxic tumour areas, where the gene product/virus is then released and acts on neighboring hypoxic tumour cells or proliferating blood vessels. In this way, the responses of macrophages to tumour hypoxia can be exploited to deliver potent anti-tumor agents to these poorly vascularized, and thus largely inaccessible, areas of tumours. Ang-2 is upregulated by blood vessels and tumour cells in many forms of tumour. It binds to the Tie2 receptor on endothelial cells and regulates vessel stabilization and angiogenesis. Recently, Tie-2+ monocytes have been shown to be recruited to experimental and human tumours where they promote tumour angiogenesis. Here we show that Ang-2 is a chemoattractant for Tie-2+ monocytes in vitro which suggests that it may help to recruit such cells into tumours. Ang-2 is upregulated by hypoxic tumour cells and macrophages upregulate Tie-2 in hypoxia. Exposure to the combined effects of Ang-2 and hypoxia suppressed the release of the pro-apoptotic cytokine, TNFalpha, and the powerful anti-angiogenic cytokine, IL-12 by macrophages. Conclusion: our data indicate that Ang-2 is capable of recruiting Tie-2+ monocytes to tumours where, together with hypoxia, it modulates their release of cytokines centrally involved in angiogenesis and metastasis.

Symposium (Mon, 24 Sep, 14:45−16:45)
Biology-driven treatment selection in head & neck cancer

47 INVITED

Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression

C. Chung. *Vanderbilt-Ingram Cancer Center, Medical Oncology, Nashville, USA*

Despite great progress in our understanding of the mechanisms that lead to cancer, there are currently no validated biomarkers of that reliably predict metastasis or survival, or whether a patient will benefit from radiation therapy. Even for targeted therapies, it is unlikely that all patients who will experience clinical benefits can be identified by a single gene analysis because of the complexity of the tumor phenotype. Technologies such as genomics and proteomics probing a large number of genes and proteins allow determining complex molecular profiles or signatures of tumors that are useful for predicting survival and treatment response. Our data suggesting that genomic and proteomic profiling can predict clinical outcome and response to therapy will be presented at the meeting. The comprehensive molecular profiles will facilitate the understanding of the tumor biology, drug mechanism and may aid proper patient selection in clinical trials; and ultimately, will improve the overall care for our cancer patients.

48 INVITED

New understanding of hypoxia-driven pathways and their potential for targeting with radiotherapy

B.G. Wouters. *Maastricht University, Radiation Oncology (Maastro), Maastricht, The Netherlands*

The majority of head and neck tumors have been demonstrated to contain areas that are poorly oxygenated. These hypoxic areas lead to treatment resistance and are implicated in promoting malignancy through changes in metabolism, angiogenesis and metastasis. Oxygen sensing pathways strongly influence both cell behavior and cell survival during hypoxia through their ability to alter gene expression, and have thus received attention as novel targets for therapy. Most research to date has focused on the HIF family of transcription factors and their target genes that are activated during moderate hypoxic conditions. We and others have identified additional oxygen-sensing pathways that affect gene expression and hypoxia tolerance by regulating mRNA translation. Hypoxia results in inhibition of mRNA translation through at least two independent mechanisms both of which provide new opportunities for biomarkers and/or therapy targets. Each of these newly discovered oxygen-sensing pathways have unique activation parameters and are likely relevant for different oxygenation patterns in tumors. I will discuss recent insight into the mechanistic basis behind the oxygen sensitivity of these pathways, their effects on hypoxia tolerance and gene expression, and their potential as new prognositic and therapeutic targets alone or in combination with radiotherapy.